Chiral discrimination by phosphorescence

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Chapter 1

Introduction

1.1 Scope

The word chirality is derived from the Greek word for hand. It emphasizes that, for humans, the left hand is a non-superimposable mirror image of the right hand. On the molecular scale, chirality is the property of an asymmetric molecule being non-superimposable on its mirror image. These mirror images are called enantiomers. Many building blocks of life, such as amino acids, carbohydrates, proteins, nucleic acids and polysaccharides, only occur in a single enantiomeric form. As a result, the behavior of a chiral substance in the chiral environment of living systems often depends on its stereochemistry. This difference in biological activity of enantiomers indicates the importance of chiral analysis in many disciplines including pharmaceutical, clinical, environmental, agricultural, and food sciences.

The development of methods for chiral analysis is extremely challenging because the chemical and physical properties of a pair of enantiomers are identical in an achiral environment. Enantiomers only differ in their interactions with other chiral compounds or with polarized light. Therefore, many chiral separations in liquid chromatography and capillary electrophoresis (CE) are based on the formation of diastereomers or diastereomeric complexes with other chiral compounds. Chiroptical detection techniques, such as polarimetry and circular dichroism, on the other hand, are based on the different response of enantiomers to polarized light.

This thesis focuses on the use of room-temperature phosphorescence in the liquid state as an enantioselective detection method. In contrast to the chiroptical detection techniques mentioned above, the difference in the phosphorescence response for two enantiomers is not based on their interaction with polarized light, but on their interaction with a chiral selector. Compared with fluorescence, phosphorescence is much more sensitive to its micro-environment, potentially resulting in a larger observed enantioselectivity in luminescence lifetimes and quantum yields.

Two main research lines can be discerned in this thesis. The first research line deals with the use of phosphorescence as a sensitive detection method in chiral cyclodextrin-based electrokinetic chromatography (CD-EKC) separations. Interestingly, the cyclodextrins used for the chiral separation can, in favorable cases, also create an enantioselective response in phosphorescence detection. The inclusion of phosphorescent enantiomers into cyclodextrins potentially results in the formation of diastereomeric complexes with different phosphorescence lifetimes, which can be used for distinction between two enantiomers. Unlike chiroptical detection techniques, which suffer from a low sensitivity, on-line coupling of enantioselective phosphorescence detection with microscale separations is very promising. Different detection schemes based on direct and indirect (sensitized and quenched) phosphorescence detection are developed and tested using different excitation sources, including a pulsed light-emitting diode, laser, and xenon lamp.

The second research line focuses on the interaction of chiral ligands with the transport protein human serum albumin (HSA). Compared to cyclodextrins, this protein is a more complex chiral selector. Although under the experimental conditions applied the HSA luminescence results only from its single tryptophan moiety, the protein contains several binding sites for ligands and can be present in several conformations in solution. Therefore, the elucidation of the stereoselective interaction of chiral therapeutic drugs with HSA is challenging. This thesis shows that time-resolved phosphorescence and fluorescence measurements can be successfully invoked to study these interactions.

1.2 Outline

Some general concepts of phosphorescence luminescence and chirality are discussed in **chapter 2**. Furthermore, a brief introductory description of cyclodextrins and human serum albumin is given. More detailed and dedicated discussions on theory are presented in the corresponding chapters.

The coupling of enantioselective phosphorescence detection to a chiral separation in CE is described in **chapter 3** for the analysis of (±)-camphorquinone. A time-resolved phosphorescence detector is built for on-line phosphorescence lifetime determination using a pulsed light-emitting diode for direct excitation of camphorquinone. Special attention is paid to the influence of the cyclodextrins used for establishing the separation on the enantioselectivity of the phosphorescence. Eventually, the method is used to determine the enantiomeric impurity in commercial standards of (+)- and (-)-camphorquinone.

The sensitivity of the enantioselective detection method can be enhanced by using sensitized phosphorescence, in which (±)-camphorquinone is indirectly excited by energy transfer from a donor reagent. **Chapter 4** describes the choice of an efficient energy donor and the influence of sensitization on the enantioselectivity in the presence of the different cyclodextrins. For the on-line phosphorescence lifetime determination, the home-built detection system is equipped with a solid-state laser for excitation of the donor. The improved sensitivity is demonstrated with the analysis of (±)-camphorquinone leaching out of a cured dental resin.

Chapter 5 treats the use of direct and sensitized phosphorescence detection coupled to the chiral separation of bupropion. In the sensitized mode, the analyte bupropion is excited and donates energy to an acceptor reagent with a high phosphorescence yield. Batch experiments directed at finding the optimal pH and cyclodextrin for direct phosphorescence detection, and the choice of acceptor for sensitized phosphorescence detection are described. The detection method is used coupled to the CE analysis of (+)- and (-)-bupropion in a pharmaceutical formulation and a spiked urine sample.

In addition to the two sensitized phosphorescence modes described in the previous chapters, quenched phosphorescence detection is a final mode of indirect phosphorescence detection. It is based on the dynamic quenching of the strong emission of a phosphorescing

reagent added to the background electrolyte. **Chapter 6** describes the optimization of the chiral analysis of the non-phosphorescent drug L-methotrexate using capillary electrophoresis and quenched phosphorescence detection. The approach is used to determine minor impurity levels of D-methotrexate and to analyze a cell culture extract spiked with L-methotrexate.

The potential of phosphorescence for elucidating the interaction of chiral drugs with the transport protein HSA is explored in **chapter 7**. The luminescence quenching of the single tryptophan moiety in HSA by a racemic mixture of the antihistamine drug brompheniramine is studied as a function of pH with steady-state and time-resolved fluorescence and phosphorescence. The heavy atom effect of iodide is used for enhancement of the phosphorescence emission. Decay-associated fluorescence spectra show the effect of the addition of iodide and brompheniramine on the emission of different protein conformations.

The binding of the separate enantiomers of the non-steroidal anti-inflammatory drug naproxen to HSA is discussed in **chapter 8**. In contrast to brompheniramine, the luminescence of naproxen itself can be detected under the experimental conditions applied. The significant differences in absorption, fluorescence, and phosphorescence of naproxen and HSA enable the selective determination of the ligand luminescence in presence of protein. Therefore, not only the influence of the binding on the luminescence of HSA is investigated, but also the effect on the luminescence of naproxen itself.

Similar to brompheniramine, the non-steroidal anti-inflammatory drug flurbiprofen cannot be selectively excited. Therefore, in **chapter 9** the stereoselective difference in HSA-binding of the flurbiprofen enantiomers and their methyl esters is investigated based on the phosphorescence of the protein's tryptophan only. In addition to the time-resolved fluorescence studies on the influence of iodide on the various HSA conformers in chapter 7, this chapter also describes the influence of iodide on the phosphorescence of HSA.

Finally, **chapter 10** provides some general conclusions and perspectives on the use of phosphorescence for chiral discrimination purposes. The results described in this thesis strongly suggest that enantioselective phosphorescence detection deserves extensive attention, especially for investigating stereoselective dynamic processes on a millisecond timescale.